Factors Related to Gastric Hypersecretion During Pregnancy and Lactation in Rats

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We attempted to elucidate the factors involved in gastric hypersecretion of rats during pregnancy and lactation. Acid secretion in pylorus-ligated and vagally denervated fistula rats stimulated with histamine, tetragastrin, and methacholine increased from midterm pregnancy and persisted during lactation. Pepsin secretion remained unaltered during pregnancy but increased during lactation. Vagal denervation itself abolished this hypersecretion. In late pregnancy, a delayed appearance of maximal acid response to histamine was apparent, as compared to nonpregnant rats, and was abolished by aminoguanidine treatment. There was a delay in the maximal response to tetragastrin but not to methacholine. Serum histamine concentrations were 3–4 times higher in late pregnancy, as compared to nonpregnant, lactating and nonlactating rats. Gastric DNA and protein concentrations were significantly increased in lactating rats with concomitant elevation of food intake and serum gastrin levels. Those changes disappeared in nonlactating rats, and gastric secretion was much the same in the nonpregnant rats. These results indicate that acid hypersecretion during pregnancy was exclusively associated with vagal innervation plus high serum histamine levels, while acid and pepsin hypersecretion in lactating rats were associated with vagal innervation plus hyperplastic gastric mucosa and high serum gastrin levels.

There is a marked gastric acid hypersecretion during pregnancy and lactation in rats (1–3). An increase of histamine-forming capacity, mobilization of mucosal histamine, hyperplasia, or hypertrophy of gastric mucosa during these periods have been given as explanations for this phenomenon (1, 4, 5). However, the secretion of pepsin during these periods was not given much attention. Our previous study showed that pepsin secretion remained unaltered in pylorus-ligated pregnant rats, despite a marked increase in acid secretion (6). In contrast, there was an apparent increase in both acid and pepsin secretion in lactating rats. Therefore, we investigated possible mechanisms related to gastric hypersecretion during pregnancy and lactation, by measuring basal and stimulated gastric secretion, serum histamine and gastrin levels, food intake, and nucleic acid and protein concentrations in the gastric mucosa of rats.

MATERIALS AND METHODS
Donryu strain rats (180–200 g) were used. A vaginal smear was made in the afternoon, and two rats in proestrous were placed in a cage with one male (300–350 g). The next morning another vaginal smear was made, and the animals in which sperm was found were considered pregnant (day 0 of pregnancy).

Gastric Secretory Study. Basal gastric secretory activity was determined in nonpregnant, pregnant (days of pregnancy 5, 10, 15, and 20), lactating (days 10 and 20), and nonlactating (days 10 and 20) rats. Rats were deprived of food but allowed free access to water for 18 hr prior to the experiment. Under ether anesthesia, the abdomen was incised and the pylorus ligated. Seven hours after pylorus
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was measured by weighing the food provided and the amount consumed. Pepsin activity was determined by Anson’s method using bovine albumin as a substrate (7) and expressed as mg tyrosine/ml. Titratable acid and pepsin output were calculated and expressed as $\mu$Eq/hr and mg tyrosine/hr, respectively.

Stimulated gastric secretory responses to secretagogues were determined in vagotomized rats, including nonpregnant, pregnant (day 20), lactating (day 10), and nonlactating (day 10). Since our objective was to obtain the data on actual increase in acid and pepsin output in response to each secretagogue during pregnancy and lactation, these experiments were performed in vagotomized rats in which basal gastric secretion was scanty. Rats were deprived of food but allowed free access to water for 18 hr prior to the experiment. Under ether anesthesia, the abdomen was incised and bilateral vagotomy was performed at the subdiaphragmatic portion. After pylorus ligation, an acute fistula was made in the foregut and a fine polyethylene cannula was inserted into the tail vein. The rats were kept in individual cages with a longitudinal slit at the bottom. After recovery from anesthesia, histamine $2\text{HCl}$ (8 mg/kg/hr), tetragastrin (125 $\mu$g/kg/hr), or methacholine bromide ($64 \mu$g/kg/hr) was infused continuously through the tail vein at a rate of 1.2 ml/hr using a peristaltic pump (Harvard Apparatus, model 931 D). The doses of stimulants were those which produce a submaximal stimulation of acid secretion in fistula rats (unpublished data). Gastric juice was collected hourly through the fistula by gravity drainage and analyzed for volume, acidity, and pepsin activity, as described above. The data were expressed as acid ($\mu$Eq/hr) or pepsin (mg tyrosine/hr) output. Only in the case of histamine was gastric secretory response determined in rats on days 1, 5, 10, and 20 of pregnancy. In half the pregnant rats subjected to histamine infusion at day 20, aminoguanidine (20 mg/kg) was given subcutaneously once daily for 4 days on days 16 to 19 of pregnancy.

**Determination of Histamine in Serum.** Serum histamine concentrations were determined before and during histamine infusion (8 mg/kg/hr) in nonpregnant, pregnant (day 20), lactating (day 10), and nonlactating (day 10) rats. Blood was collected from the descending aorta and centrifuged at 3000 rpm for 30 min at 4°C. Serum histamine concentrations were determined by the fluorometric method of Shore et al (8). Briefly, the method involves extraction of histamine into butanol from alkalized perchloric acid serum extract, returning to an aqueous phase, and the highly fluorescent product is then estimated on a Hitachi spectrofluorometer (activation 360 nm, fluorescence 450 nm). Results were expressed as $\mu$g histamine/ml serum.

**Determination of Food Consumption, Serum and Antral Gastrin Levels, and Nucleic Acids and Protein Concentrations.** Rats were housed individually in cages with a solid bottom containing sawdust as bedding material. Purina chow was provided *ad libitum*, and the amount consumed was measured by weighing the food provided and the spilled food during the experimental period. At days 7, 15, and 20 of pregnancy and days of 10, 15, and 20 of lactation, blood was collected from the heart of ether-anesthetized rats. The stomach was removed, rinsed in cold saline, and opened along the greater curvature. Antral tissue was dissected out, weighed, and homogenized in 5 ml of cold, deionized water and boiled in a water bath for 20 min. After centrifugation of blood and tissue samples, the supernatants were stored at $-20°C$. The oxyntic mucosa was scraped off from the corpus and approximately 200 mg of mucosa was used for *in vitro* measurement of nucleic acids and protein concentrations. RNA was extracted by incubating the tissue samples for 90 min at 37°C with 0.3 N KOH. DNA was extracted with perchloric acid for 20 min at 70°C, and protein was solubilized by 1 ml of NaOH. The concentrations of RNA were measured according to the orcinol reaction (9), of those of RNA and protein according to Lowry et al (11). Results were expressed as $\mu$g/100 mg of wet tissue for RNA and DNA and as mg/100 mg of wet tissue for protein. The concentrations of serum and antral gastrin were determined by radioimmunoassay (12), and results were expressed as pg/ml of serum or $\mu$g/g of antral tissue, respectively.

**Drugs.** Drugs used were histamine $2\text{HCl}$ (Nakarai), tetragastrin (Sumitomo), methacholine bromide (Tokyo Kasei), and aminoguanidine sulfate (Sigma). All drugs were dissolved in saline before use.

**Statistics.** Means were compared by Student’s *t* test for unpaired values and were considered to be significantly different if $P < 0.05$.

**RESULTS**

**Basal Secretion.** Gastric secretion in pylorus-ligated rats increased during pregnancy and lactation (Figure 1). The volume and acid outputs were 2 or 3 times greater than those in nonpregnant rats on day 20 of pregnancy or day 10 of lactation, respectively. Pepsin activity was reduced gradually during pregnancy but returned to the levels seen in nonpregnant rats at day 10 of lactation. However, pepsin output significantly increased in parallel with acid output during lactation. In rats from which pups had been removed on the day of delivery (nonlactating rats), there was no further increase in gastric secretion. The values were much the same as those in the nonpregnant rats within 10 days after parturition. When the amount of gastric juice was plotted versus time in the four groups, the infusion of histamine, tetragastrin, or methacholine significant-